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## The Effect of Pasak bumi (*Eurycoma longifolia* Jack) Ethanol Extract on the Lipid Profile of the Rats Was Induced High-Calorie High Fat Diet

Triawanti<sup>1</sup>, Didik Dwi Sanyoto<sup>2</sup>, Azma Rosida<sup>3</sup>, Hendra Wana Nur'amin<sup>4</sup>,  
Gastin Gabriel Jangkang<sup>5</sup>, Dimas Ikhsan Airlangga<sup>6</sup>

<sup>1</sup>Department of Biochemistry and Biomolecular Medical Faculty of Lambung Mangkurat University, Indonesia

<sup>2</sup>Anatomy Division of Biomedic Department, Medical Faculty of Lambung Mangkurat University, Indonesia

<sup>3</sup>Department of Clinical Pathology, Medical Faculty of Lambung Mangkurat University, Indonesia

<sup>4</sup>Department of Pharmacology, Medical Faculty of Lambung Mangkurat University, Indonesia

<sup>5</sup>Student of Medical Faculty, Lambung Mangkurat University, Indonesia

<sup>6</sup>Student of Medical Faculty, Brawijaya University, Indonesia

### Abstract:

**Background:** Obesity is often followed by hyperlipidemia which can increase morbidity and metabolic syndrome. Therapy is needed that is able to overcome not only obesity but also a metabolic syndrome that occurs. The saponin content in the pasak bumi is thought to reduce the absorption of dietary fat, tannin can inhibit the activity of lipoprotein lipase and glycerolphosphate dehydrogenase so that it can overcome hyperlipidemia. This study aims to prove the effect of ethanol extracts of pasak bumi (EPB) on the lipid profile of rats induced by a high-fat diet.

**Materials and Methods:** The research was an experimental study. Rats were divided into 6 groups: (K0) normal mice + placebo; (K1) overweight mice + placebo (aquades); (P1) overweight mice + EPB 7.5 mg/kgBW; (P2) overweight mice + EPB 15 mg/kgBW; (P3) overweight mice + EPB 22.5 mg/kgBW; (P4) overweight mice + EPB 30 mg/kgBW. Ethanol extract of 70% pasak bumi is given for 4 weeks. Then the lipid profile is examined. Analysis of data using the Oneway Anova test with a confidence level of 95%.

**Results:** There were significant differences in cholesterol, triglyceride, and LDL levels in the treatment group. The group given EPB 7.5 mg/kgBW had lower cholesterol and LDL levels compared to other groups, while the lowest triglyceride levels in the group given EPB was 15 mg/kgBB. There were no differences in HDL levels in all treatment groups.

**Conclusion:** Ethanol extract pasak bumi 7.5 and 15 mg/kg body weight can reduce cholesterol, LDL, and triglyceride levels of rats induced by high-fat diets.

**Key Word:** obesity, hyperlipidemia, lipid profile, pasak bumi (*Eurycoma longifolia*. Jack)

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### I. Introduction

Obesity occurs due to an imbalance between energy intake and expenditure. The etiology of obesity involves various biological components, genetic and environmental factors. Thus obesity becomes a condition that is not easy to treat because of a complex etiology. As a consequence, there are very few drugs that are able to cure obesity, its mechanism of action is difficult to understand and side effects are generally serious. Pharmacologically the basis of antiobesity drugs can be grouped into 4 namely ingredients that primarily reduce appetite through central work, ingredients that primarily increase metabolic speed or affect metabolism through peripheral work, materials that work on the gastrointestinal tract, materials that not only affect obesity, but also all metabolic syndrome<sup>1</sup>.

Hyperlipidemia often follows the incidence of obesity. The increase in obesity likely results from a complex interaction between all risk factors<sup>2</sup>. These conditions will cause an increase in morbidity in obesity. Obesity can reduce life expectancy, impaired quality of life, and also disabilities with a higher risk in those individuals who develop cardiovascular diseases and type 2 diabetes<sup>3</sup>. Even if drugs for obesity exist, it can cause all sympathomimetics stimulant-like adverse such as insomnia, increases in heart rate and blood pressure<sup>4</sup>. Hyperlipidemia causes an increase in oxidative stress and chronic inflammation which is a mechanism for the emergence of various complications<sup>1</sup>. Thus the management of hyperlipidemia in obesity is one of the main concerns.

In the study of Gitawati et al<sup>5</sup> the use of herbal medicine in dyslipidemia patients was 62.9% with the highest content of Simplicia, namely Dutch teak leaves (*Guazumaefolium*) and yellow leaves (*Murrayae folium*). Research by Demjan et al<sup>6</sup> on the effect of *Stellaria media* tea on the lipid profile of Wistar rats showed that there was no significant difference in total cholesterol, LDL, HDL levels between groups given *Stellaria media* and the control.

Pasak bumi (*Eurycoma longifolia* Jack) is a hardy plant that grows endemic in Indonesia. Pasak bumi become commonly used as herbal medicine by Indonesian people<sup>7</sup>. Pharmacological studies have shown that Pasak Bumi can be used for exhibited anti-malarial, anti-cancer, anti-inflammatory, and other effects that have not to found yet<sup>8</sup>. Pasak bumi contains active substances, namely saponins, tannins, polyphenols, and xanthin. It also has main components such as eurycomanone, quassinoids, coumarin, and glikosiad<sup>9</sup>. Saponins can reduce the absorption of dietary fat, as demonstrated by saponins in the *Platycodon grandiflorum* A.De plant<sup>10</sup>. Tannin can inhibit the activity of lipoprotein lipase and glycerophosphate dehydrogenase, as well as tannin in *Salacia reticulata* plants<sup>11</sup>. In addition, as has been reported by several researchers, the content of polyphenols (flavonoids) in some plants can cause apoptotic effects of fat cells<sup>12</sup>.

Until now have been no studies on the effect of pasak bumi on the lipid profile in mice fed a high-calorie high-fat diet. The results of a systematic review by Rouhi-Boroujeni et al<sup>13</sup> show that ginseng plants can reduce serum triglyceride, total cholesterol, LDL-C, and increase HDL-C. The content of some of the compounds that are similar to the ginseng plant in the pasak bumi is expected to improve the lipid profile in rats fed a high-fat, high-calorie diet. This study aims to prove the effect of giving 70% ethanol extracts of pasak bumi to the lipid profile of rats induced by a high-calorie and fat diet.

## II. Material And Methods

**Research design:** This study has received ethical approval from the Research Ethics Commission of the Faculty of Medicine at Lambung Mangkurat University (No. 297 / KEPK-FKUNLAM / VII / 2019). An experimental research design using *Rattus norvegicus* white mice induced by high-calorie high-fat feed. Groups of experimental animals that have been obese are divided into 6 groups, namely: (K0) normal mice + placebo; (K1) overweight mice + placebo (aquades); (P1) overweight mice + Pasak Bumi extract (EPB) 7.5 mg/kgBW; (P2) overweight mice + EPB 15 mg/kgBW; (P3) overweight mice + EPB 22.5 mg/kgBW; (P4) overweight mice + EPB 30 mg/kgBW.

**Material:** Pasak bumi, high-calorie high-fat feed, aquadest, 70% ethanol, cholesterol assay kit, Triglicerida assay kit, LDL assay kit by Roche®.

**Procedure:** Induction of experimental animals with a high-calorie high-fat diet. In this study, rats were induced with a high-calorie high-fat diet to increase body weight for 12 weeks<sup>8</sup>.

**Administration of ethanol extract pasak bumi (EPB):** Once the rat were overweight, they were divided into 5 groups, namely positive control (K1): overweight rat + placebo + standard feed; (P1): overweight rat + 70% ethanol extract Pasak bumi (EPB) 7.5 mg/kg + standard feed; (P2): overweight rat + EPB 15 mg/kg + standard feed; (P3): overweight rat + EPB 22.5 mg/kgBB + standar feed; (P4): overweight rat + EPB 30 mg/kgBB + standar feed for 4 weeks; plus 1 negative control group (K0) that is healthy rat given placebo and standard feed for 4 weeks.

**Lipid profile assay:** Examination of total cholesterol, triglycerides, and Chol-HDL using the colorimetric method. Inspection procedures such as those in the Roche® manufacturer's manual. For Chol-LDL levels using the indirect method that is using the Friedewald formula based on the results of total cholesterol, Chol-HDL, and TG tests.

Friedewald's formula : Chol-LDL = Cholesterol total – (Chol-HDL + TG/5).

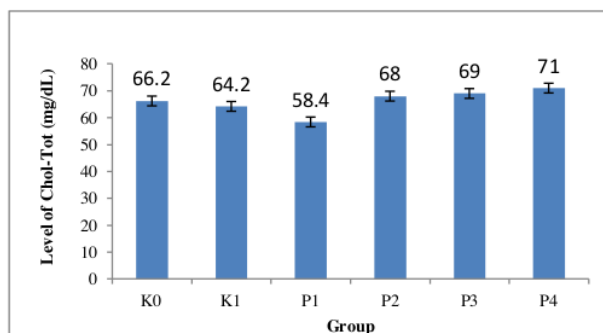
**Table 1.** Calorie and fat content in normal and high-calorie high-fat diets (modified from Tsalissavrina et al<sup>14</sup>)

| Ingredients                  | amount normal diet | Amount high-calorie high-fat diet |
|------------------------------|--------------------|-----------------------------------|
| Confeed pars (gr)            | 200                | 200                               |
| wheat flour (gr)             | -                  | 100                               |
| cholesterol (gr)             | -                  | 8                                 |
| from egg yolk of a duck      |                    |                                   |
| pig oil (gr)                 | -                  | 20                                |
| water                        | adjust             | adjust                            |
| Calorie content per 100 gram | 305 kcal           | 631 kcal                          |
| Fat content per 100 gram     | 5 gram             | 30,5 gram                         |

**Data analysis:** Data were analyzed using the Shapiro Wilk normality test and the Lavene homogeneity test. Then proceed with the One way Anova test and LSD follow-up test with a confidence level of 95%.

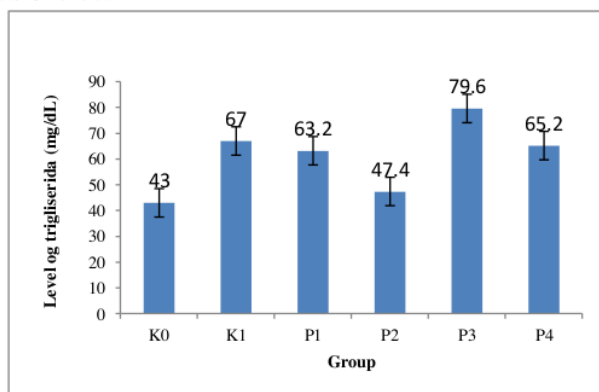
### III. Result

After giving a high-fat diet the cholesterol level of the rat became an average of 220 mg / dL and the average body weight of 277 grams. After being given peg earth extract for 4 weeks the results are presented in Figure 1-4



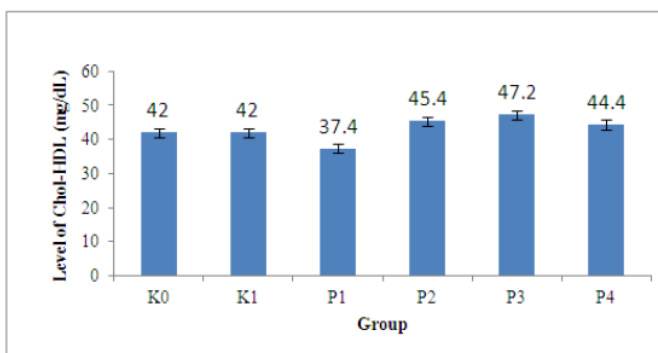
**Figure 1** Average total cholesterol levels after administration of ethanol extracts of pasak bumi (K0 = negative control, K1 = positive control, P1 = EPB 7.5 mg / kgBW; P2 = EPB 15 mg / kgBW; P3 = EPB 22.5 mg / kgBW; P4 = EPB 30 mg / kgBW; p = 0.016)

Figure 1 shows that after administration of pasak bumi extract and standard diet, cholesterol level decreased. Oneway Anova test results showed the results of p = 0.016 which means there are differences between treatment groups. Figure 1 shows that the total cholesterol level in group P1 given EPB 7.5 mg / kgBW showed the lowest total cholesterol level. Further test results showed that there were differences between K0 and P1, P1 with P2, P3, and P4. This shows that ethanol extract of Pasak bumi with a dose of 7.5 mg / kgBW can reduce total cholesterol levels.



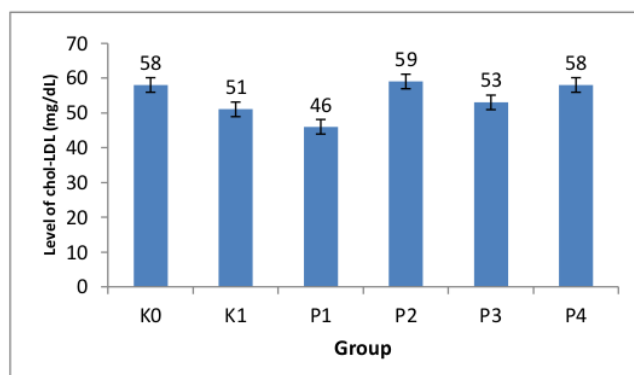
**Figure 2** Average triglyceride levels after administration of ethanol extracts of Pasak bumi (K0 = negative control, K1 = positive control, P1 = EPB 7.5 mg / kgBW; P2 = EPB 15 mg / kgBW; P3 = EPB 22.5 mg / kgBW; P4 = EPB 30 mg / kgBW; p = 0.015)

Figure 2 shows that after administration of Pasak bumi extract and standard diet, triglyceride levels were decreased. Oneway Anova test results show the results of p = 0.015 which means there are differences between treatment groups. Figure 2 shows that triglyceride levels in the P2 group given EPB 15 mg / kgBW showed the lowest triglyceride levels. Further test results showed that there were differences between K0 and K1, K0 with P3 and P4; P2 with P3. When compared between groups K1 with P2, it was seen that P2 showed lower triglyceride levels. This shows that the ethanol extract of Pasak bumi with a dose of 15 mg / kgBW can reduce triglyceride levels.



**Figure 3** Average chol-HDL levels after administration of ethanol extracts of pasak bumi (K0 = negative control, K1 = positive control, P1 = EPB 7.5 mg / kgBW; P2 = EPB 15 mg / kgBW; P3 = EPB 22.5 mg / kgBW; P4 = EPB 30 mg / kgBW; p = 0.071)

Figure 3 shows that after administration of Pasak bumi extracts and standard diets there was a decrease in Chol-HDL levels. Although the results of the Oneway Anova test showed a result of p = 0.071 which means there was no difference between the treatment groups, in Figure 3 it was seen that in the P1 group given EPB 7.5 mg / kgBB showed the lowest Chol-HDL levels. This shows that ethanol extract of pasak bumi with a dose of 7.5 mg / kgBB tends to reduce Chol-HDL levels.



**Figure 4** Average chol-LDL levels after administration of ethanol extracts of Pasak bumi (K0 = negative control, K1 = positive control, P1 = EPB 7.5 mg / kgBW; P2 = EPB 15 mg / kgBW; P3 = EPB 22.5 mg / kgBW; P4 = EPB 30 mg / kgBW; p = 0.029)

Figure 4 shows that after administration of Pasak bumi extract and standard diet, there was a decrease in Chol-LDL levels. Oneway Anova test results show the results of p = 0.029 which means there are differences between treatment groups. Figure 4 shows that the Chol-LDL levels in the P1 group given EPB 7.5 mg / kgBB showed the lowest Chol-LDL levels. The results of further tests showed that there were differences between K0 and P1, P1 and P2. When compared between groups K1 and P1, it was seen that P1 showed lower chol-LDL levels. This shows that ethanol extract of Pasak bumi with a dose of 7.5 mg / kgBB can reduce chol-LDL levels.

#### IV. Discussion

The 70% ethanol extract used in this study contained active compounds namely 8.730% saponin, 14.468% alkaloids, flavonoids 21.5 mg/mL, steroids 42.285 mg / mL, terpenoids 244.3 mg/mL and tannins 2.329 mg/mL. Based on the active compound content, the most dominant ingredients are terpenoids and steroids. Steroid compounds play a role in the formation of the hormone testosterone. The content of tannin compounds in the earth peg is thought to inhibit the activity of lipoprotein lipase and glycerolphosphate dehydrogenase which affect fat metabolism and synthesis of triacylglycerol in fat cells. Triacylglycerol synthesis is catalyzed enzyme called acyl-CoA diacylglycerol acyltransferase (DGAT)<sup>15</sup>.

High-calorie high-fat feed (HCHF) made and given for 12 weeks contains as many as 631 kcal calories/100 grams of feed and fat as much as 30.5 grams/100 grams of feed. This content is more than the standard feed given to the control group is 305 kcal and 5 grams of fat per 100 grams of feed (Table 1). High-calorie and high-fat (HCHF) feed used in this study can increase blood cholesterol. The mean blood cholesterol level of rats that were given HCHF was 220 mg/dL. The results showed that after administration of ethanol extracts of pasak bumi and standard diets were able to reduce cholesterol, triglyceride, and LDL levels close to the normal rat group.

The saponin content in the Pasak bumi is thought to reduce the absorption of dietary fats, as demonstrated by saponins in the *Platycodon grandiflorum* A.De. Saponin is isolated from medicinal plants as their main source for preparation and naturally occurring bioorganic molecule with high molecular weight (aglycone)<sup>16</sup>. Administration of methanol extract of platycodin saponin (PS) for 4 weeks in rats with a high-fat diet showed the results of a decrease in LDL cholesterol, a reduction in calorie intake that is closely related to weight loss. Platycodin saponin (PS) also can enhance skeletal muscle protein synthesis and mitochondrial function<sup>17</sup>. The mechanism of action of PS is almost the same as that of orlistat which inhibits lipase and excretes fat through feces. This potential is not only caused by one active compound but each component or combination of PS can be used as a lipase inhibitor<sup>4</sup>.

Research conducted by Jang et al<sup>18</sup> namely the administration of isoflavone-free peptide extract (BSP) taken from black soy protein in obese rats induced by diet for 13 weeks without exercise and 8 weeks of exercise. After 13 weeks, white fat mass, plasma leptin concentration, total cholesterol, and triglycerides increased in rats fed a high-fat diet. The results showed a decrease in body weight in mice given BSP compared to controls. With high doses of BSP, a decrease in serum triglyceride concentration and an increase in adiponectin concentration are obtained. Average white fat tissue and leptin concentration tend to be lower with increasing BSP content, but this tendency is not significant. From the results of his study, it was concluded that the effect of BSP is related to STAT3 activation which depends on JAK2 and direct activation of AMPK.

Pasak bumi contains polyphenols which are thought to play a role in lowering triglyceride levels in rats induced by a high-fat diet. Dietary fat is an energy-dense nutrient that gives essential fatty acids (FAs) and allows the absorption efficiency of fat-soluble vitamins<sup>19</sup>. Polyphenols act as organic compounds that are found abundantly in plants<sup>20</sup>. In the gastrointestinal tract (GIT), phenolic molecules can interact with lipid peroxidation products resultants in the digestive process<sup>21</sup>.

One of the polyphenol compounds whose effects are the most studied is the catechin contained in tea. One type of catechin in green tea is epigallocatechin gallate (EGCG). EGCG regulated lipid catabolism through AMPK, ATGL and SREBP1-c mediated downstream mechanisms, increasing lipolysis and suppressing lipogenesis that EGCG in adipocytes<sup>21</sup>. The results of the study Wolfram et al<sup>23</sup> using TEAGIVOR, a product that provides pure EGCG, concluded that in C57BL / 6J mice, EGCG supplements prevented weight gain and plasma glucose, triglyceride, and leptin levels after eating.

The tannin content in the earth peg is thought to also be able to inhibit the activity of glycerophosphate dehydrogenase, as well as tannin in *Salacia reticulata* plants<sup>11</sup>. Tannins are one of the most natural, non-toxic, and highly reactive classified abundant polyphenols aromatic biomolecules<sup>24</sup>. In lipid metabolism, glycerol-3-phosphatidehydrogenase catalyzes the reaction of triacylglycerol formation from dihydroxyacetone phosphate in adipose tissue. Increased synthesis of triacylglycerol in adipose tissue plays an important role in the development of obesity. Previous research reports that GPDH activity and body mass index have a positive correlation. In an obese state where an energy imbalance results in massive fat cell hypertrophy through increased synthesis and storage of triacylglycerol. This supports that increased GPDH activity contributes to obesity through the supply of glycerol-3-phosphate as a substrate for the synthesis of triacylglycerol<sup>1</sup>.

The results of the review by Ji Xi et al<sup>25</sup> on bioactive compounds from herbal medicines to manage dyslipidemia suggest a mechanism by which herbal medicine can inhibit cholesterol absorption in the intestine (Figure 5). Bioactive compounds from herbal medicines could regulate these processes. (A) Regulation of NPC1L1, including curcumin, lycopene, monascin, and ankaflavin. (B) Triterpenic acid (oleanolic acid (OA) and ursolic acid (UA)) and berberine. (C) Taxifolin, quercetin, naringenin, hesperetin, and tangeretin. (D) Tangeretin, nobiletin, and lignin (E) Curcumin, lycopene, camphene<sup>25</sup>. The small intestine absorbs lipids exogenously by the diet and endogenously by the enterocytes shed from the intestinal mucosa<sup>19</sup>. In this mechanism, it is explained that diet and bile enter the intestinal cells via the Niemann-Pick C1 like 1 transmembrane protein (NPC1L1) from the lumen to the membrane brush border enterocytes. Niemann-Pick type C (NPC) is characterized by the accumulation of cholesterol, sphingomyelin, and other lipids in endosomes and lysosomes<sup>26</sup>. In enterocytes, free cholesterol (FC) is esterified into cholesterol esters (CEs) by acyl CoA: cholesterol acyltransferase (ACAT) -2 in the endoplasmic reticulum (ER). Furthermore, CEs and triglycerides under the action of microsomal triglyceride transfer protein (MTTP) form chylomicrons which are secreted into the lymphatic system. Microsomal triglyceride transfer protein (MTP) is important for lipid metabolism, especially in the biogenesis of very low-density lipoproteins and chylomicron<sup>27</sup>. In addition through the

inhibition of cholesterol absorption in the intestine, medicinal plants also can suppress cholesterol synthesis, promotion of reverse cholesterol transport, acceleration of excretion of cholesterol in the liver<sup>25</sup>.

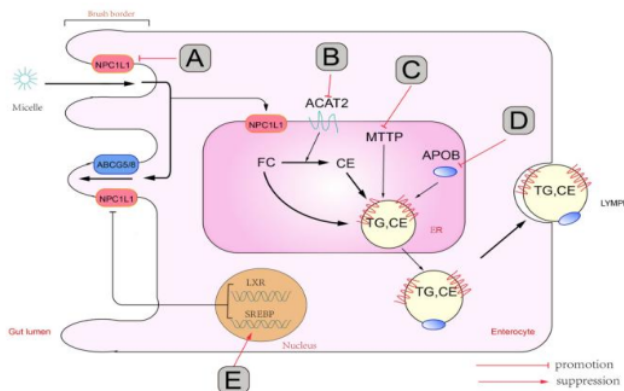


Fig. 5. Herbal medicine inhibits cholesterol absorption<sup>25</sup>

## V. Conclusion

Based on the results of the study it can be concluded that administration of ethanol extracts of Pasak bumi doses of 7.5 and 15 mg/kgBW can improve the lipid profile of rats induced by a high-fat diet, whereas at higher doses of 22.5 and 30 mg/kgBW do not show significant differences.

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